Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2014) **15**(7), 747–761 DOI: 10.1111/mpp.12127

Review

Ergosterol, an orphan fungal microbe-associated molecular pattern (MAMP)

ROBYN L. KLEMPTNER†, JOHN S. SHERWOOD†, FIDELE TUGIZIMANA†, IAN A. DUBERY AND LIZELLE A. PIATER*

Department of Biochemistry, University of Johannesburg, Johannesburg 2006, South Africa

SUMMARY

Fungal pathogens continue to pose a significant threat to crop production and food supply. The early stages of plant–fungus interactions are mostly mediated by microbe-associated molecular pattern (MAMP) molecules, perceived by plant pattern recognition receptors (PRRs). Currently, the identified fungal MAMP molecules include chitin, chitosan, β-glucans, elicitins and ergosterol. Although the molecular battles between host plants and infecting fungal phytopathogens have been studied extensively, many aspects still need to be investigated to obtain a holistic understanding of the intrinsic mechanisms, which is paramount in combating fungal plant diseases. Here, an overview is given of the most recent findings concerning an 'orphan' fungal MAMP molecule, ergosterol, and we present what is currently known from a synopsis of different genes, proteins and metabolites found to play key roles in induced immune responses in plant–fungus interactions. Clearly, integrative investigations are still needed to provide a comprehensive systems-based understanding of the dynamics associated with molecular mechanisms in plant–ergosterol interactions and associated host responses.

Keywords: ergosterol, fungal pathogens, genomics, MAMPs, metabolomics, plant–fungus interactions, proteomics.

APOCALYPSE NOW: FUNGAL PHYTOPATHOGEN WARFARE

Global losses of economically important crop plants as a result of the destructive interaction of virulent fungi and their respective elicitors/effectors with plant hosts have always been a major concern to food yield and security, as well as to the maintenance of biodiversity within ecosystems (Fisher *et al*., 2012; Sharma *et al*., 2011). Despite this fact, there is a substantial short-fall in information on specific plant–fungus interactions when compared with the vast number of investigations into plant–bacterium interactions. Thus, as a result of the increase in emerging infectious diseases (EIDs) during the 21st century, research in this regard has intensified worldwide (Pennisi, 2010). Indeed, it is estimated that up to 70% of global EIDs affecting all kingdoms of life are caused either directly or indirectly by fungal pathogens (Fisher *et al*., 2012).

The up to 125 million tonne loss in the five most economically important food crops (potatoes, maize, rice, wheat and soybean) represents the potentially devastating current-day impact of fungal pathogens on both food security and economic stability. This contributes greatly to the promotion of poverty and financial loss within global agricultural sectors. Losses occurring as a result of maize, rice and wheat diseases exclusively total nearly $60 billion per year; thus, if the spread of these diseases and those affecting other plants can be managed more effectively, up to 600 million people could be fed through increased food yield (Fisher *et al*., 2012). This showcases the extensive impact of fungal pathogens on modern food security, and thus the integrity of agricultural industries must be maintained effectively to curb crop losses as much as possible (Fisher *et al*., 2012). Thus, an understanding of the molecular mechanisms involved in plant–fungus interactions is of paramount importance, as this would open up the possibility to develop novel, more effective and sustainable strategies to control or eradicate fungal diseases in plants. To set the terrain for the current combat scene, a patrolled mission on general background information and plant–fungus interactions will first be surveyed before delving into specific plant–ergosterol interactions.

INNATE IMMUNITY: THE CENTRAL COMMAND UNIT TO COORDINATE PLANT DEFENCE

The continuing biological war between plants and various microbial pathogens, as with all co-evolving organisms, for survival of the fittest, is inescapable (Bittel and Robatzek, 2007). To defend themselves against pathogens, plants utilise a protection system (passive resistance) that involves an array of structural barriers and pre-formed antimicrobial phytoanticipins to prevent/attenuate invasion by potential attackers (Pritchard and Birch, 2011; Schwessinger and Zipfel, 2008). However, this protection

**Correspondence*: Email: lpiater@uj.ac.za
†These authors contributed equally and are listed alphabetically.

system can be successfully overcome by numerous phytopathogens to ultimately cause infection and disease. Lacking an adaptive immune system, plants may then initiate dynamic innate immune defences that are activated once danger signals have been perceived.

The molecular battles occurring between host plants and their infecting phytopathogens have been fascinating fields of research for decades (Jones and Dangl, 2006; Pieterse *et al*., 2009). Since the 1940s, scientists have been trying to unravel the complex molecular mechanisms that comprise the plant innate immune system (Flor, 1942; Thomma *et al*., 2011). The notion of these immune responses and their interactions with the pathogens causing plant diseases is far more complex, and has been revised and re-formulated many times. The conception of the initial gene-for-gene resistance hypothesis (Flor, 1942; Keen, 1990), the more specific characterisation and grouping of various pathogenic signature molecules into different classes of elicitors and effectors (Bent and Mackey, 2007; Boller and Felix, 2009), the coinage of the interrelated 'zig-zag hypothesis' terms microbe/pathogen-associated molecular pattern (M/PAMP)-triggered immunity (M/PTI) and effector-triggered immunity (ETI) (Jones and Dangl, 2006), and the concepts of systemic acquired resistance (SAR) (Van der Biezen and Jones, 1998) and induced systemic resistance (ISR) have all contributed greatly to our understanding and elucidation of this multicomponent immune system of plants (Fig. 1). It should be noted that, from here onwards, the term MAMP is preferred to PAMP so as to be more inclusive. Until recently, these concepts and pathways have largely been treated as separate entities and studied in an isolated sense; however, recent reports have suggested a more intricate interplay and continuity between defence pathways, which ultimately culminate in a single, complex interconnected signalling network deployed by the plant as necessary (Boller and Felix, 2009; Katagiri and Tsuda, 2010; Qi *et al*., 2011; Thomma *et al*., 2011; Vidhyasekaran, 2014).

The basic concept of immunity and host resistance remains largely the same. In order for the successful establishment of a phytopathogen within a potential host plant, initial avoidance and traversing of the host's pre-formed defence responses are essential (Deepak *et al*., 2006; Fan and Doerner, 2012; Gonzalez-Lamothe *et al*., 2009; Madala *et al*., 2011; Sanabria *et al*., 2010). During the process certain conserved molecular signatures/motifs present in a particular class of microbes (MAMPs) may be specifically recognised by dedicated host receptors (pattern recognition receptors or PRRs), thereby initiating inducible defence response cascades (Beck *et al*., 2012; Henry *et al*., 2012; Sanabria *et al*., 2010; Zipfel, 2008,2009), resulting in immunity through MTI activation (Jones and Dangl, 2006). It should also be noted that wounding or pathogen attack may lead to the production of damage-associated molecular patterns (DAMPs), which are categorised and recognised in a similar fashion to MAMPs. From here, the paradigm of MTI and ETI, or the zig-zag hypothesis of plant–pathogen interactions and plant defences, originated (Fig. 1). In this regard, MAMPs can be described as general elicitors of the basal defence response. Bacterial MAMPs include flagellin (Zipfel, 2008), EF-Tu (elongation factor-thermounstable) (Postel and Kemmerling, 2009), LPS (lipopolysaccharide) from Gram-negative bacteria (Gerber and Dubery, 2004; Gerber *et al*., 2006) and peptidoglycan from Gram-positive bacteria (Nürnberger *et al*., 2004), whereas fungal MAMPs include chitin (Granado *et al*., 1995), chitosan (Amborabé *et al*., 2008), xylanase (Hou *et al*., 2013), elicitin proteins (Yang *et al*., 2012), cerato-platanin proteins (Frías *et al*., 2013) and the sterol ergosterol (Rossard *et al*., 2010).

ATTACK AND DEFENCE: FUNGUS *VERSUS* PLANT

Induced responses ultimately require the activation of many complex cascades, and one of the initial responses to ensue following MAMP perception and host infection is transcriptional reprogramming of the host cell transcriptome (Vidhyasekaran, 2014), with downstream protein expression, signal transduction and the production of numerous secondary metabolites, including phytoalexins. Although the molecular mechanisms of these induced immune responses are not yet fully understood, the general plant defence reaction can be simplified into interconnected steps: following the perception of the non-self/altered-self/damaged-self entities (Sanabria *et al*., 2010), the early cellular events include changes in plasma membrane permeability, the influx of Ca^{2+} and H^+ and efflux of Cl^- and K^+, pH changes, plasma membrane depolarisation at the immediate site of infection, generation of reactive oxygen and nitrogen species (ROS and RNS), ion-induced signalling, mitogen-activated protein (MAP) kinase activation and accumulation of pathogenesis-related (PR) proteins (Fig. 1) (Almagro *et al*., 2009; Arasimowicz and Floryszakwieczorek, 2007; Lecourieux *et al*., 2006; Ma, 2011; Piater *et al*., 2004; Vatsa *et al*., 2011a, b). The molecules of these cellular events can even circulate to distal parts of the plant, giving rise to SAR (Mishina and Zeier, 2007) and an associated increase in the levels of phytohormones, thereby effectively controlling the spread of the pathogen through the priming of uninfected plant parts and destruction of infected plant tissues.

However, many aspects of the molecular mechanisms involved in plant–pathogen interactions are still unknown. Transcriptomic- and proteomic-based studies have provided tremendous knowledge about the plant immune response, but with certain limitations. Metabolomics is now being suggested as the ultimate level of post-genomic analyses, as it provides a comprehensive picture of the molecular organisation of an organism under defined biological conditions, thus reflecting both transcriptional and post-transcriptional regulation (Allwood *et al*., 2008, 2010; Tugizimana *et al*., 2013). Plant–microbe interactions can thus be

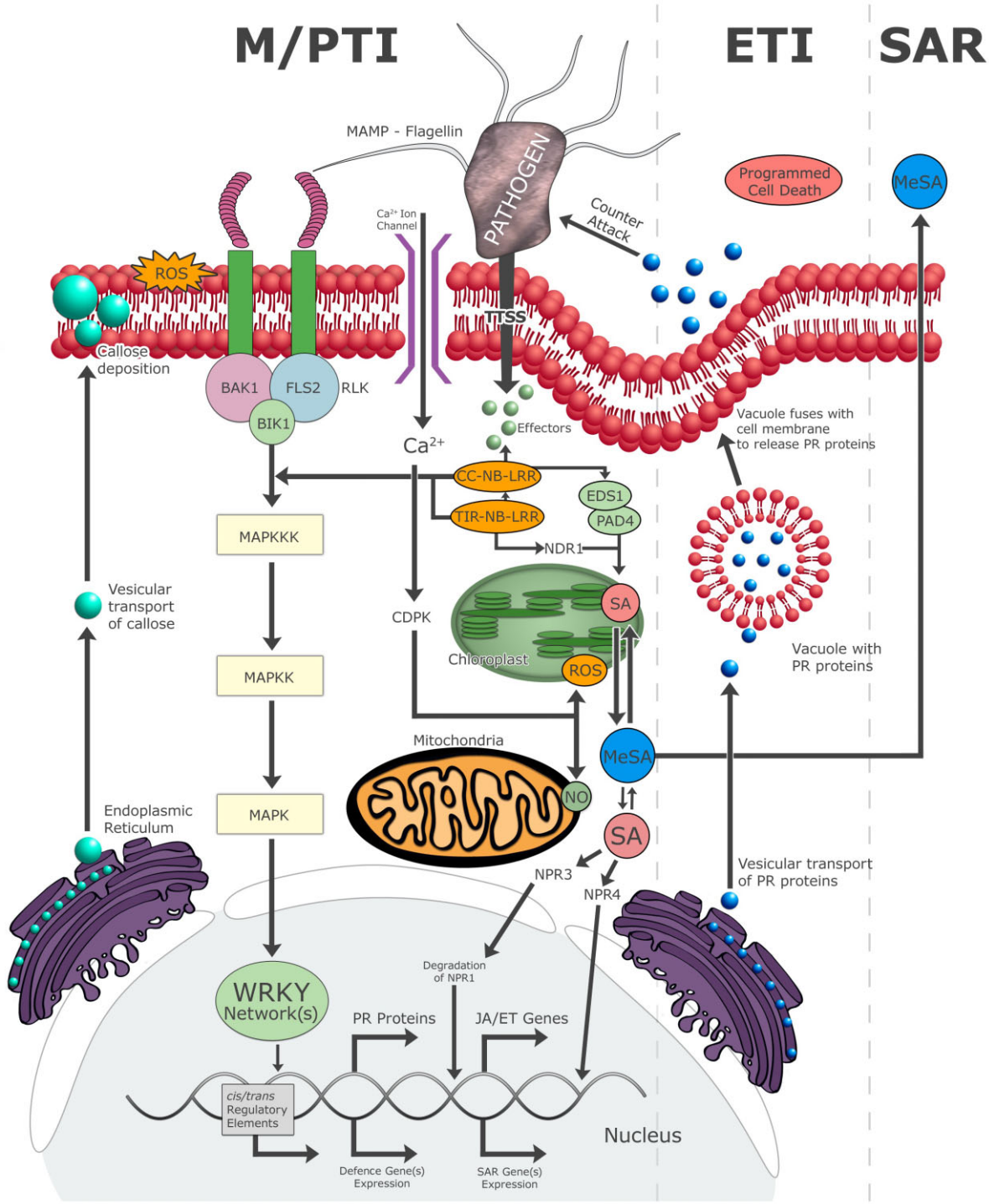


Fig. 1 Diagram illustrating the interplay between plant innate immune responses and cellular events with regard to microbe/pathogen-associated molecular pattern (M/PAMP)-triggered immunity (M/PTI), effector-triggered immunity (ETI) and the concomitant hypersensitive response (HR)/programmed cell death (PCD), and systemic acquired resistance (SAR) (adapted from Muthamilarasan and Prasad, 2013). BAK1, brassinosteroid insensitive 1 (BRI1)-associated kinase; BIK1, *Botrytis*-induced kinase 1; CC-NB-LRR, coiled-coil nucleotide-binding leucine-rich-repeat; CDPK, calcium-dependent protein kinase; EDS1, enhanced disease susceptibility 1; ET, ethylene; FLS2, flagellin-sensitive 2; JA, jasmonic acid; MAPK(K/K), mitogen-activated protein kinase (kinase/kinase); MeSA, methyl-SA; NPR, nonexpressor of PR gene; PAD4, phytoalexin deficient 4; PR, pathogenesis-related; RLK, receptor-like kinase; ROS, reactive oxygen species; SA, salicylic acid; TIR, Toll and Interleukin 1 transmembrane receptor; TTSS, type III secretion system.

described as a co-evolving molecular war between pathogen and host, with specialised weaponry available to both partners that can be activated and used as required, and the continuous competition between the opponents leads to successful infection by the pathogen or effective resistance in the host.

ERGOSTEROL: BETRAYING THE PRESENCE OF FUNGAL ATTACKERS

Ergosterol, a 5,7-diene oxysterol, is the most predominant sterol found in fungal cell membranes (Mohd As'wad *et al*., 2011; Zhao *et al*., 2005) and is of particular interest as a MAMP because of its potential to activate lipid-based signalling events. Ergosterol biosynthesis has never been reported in plants and is thus recognised as 'non-self' by a plant cell, thereby falling into the lipophilic class of biotic elicitors (Angelova *et al*., 2006; Granado *et al*., 1995; Sanabria *et al*., 2010). Although *in planta* determination of ergosterol can be used to estimate the level of fungal infection (Mohd As'wad *et al*., 2011), i.e. as a fungal marker as documented in cereal crops, including barley and corn (Dohnal *et al*., 2010), to date, this MAMP has not received as much attention as would be expected.

Sterols are part of the vast family of isoprenoid compounds, and display both chemical complexity and remarkable functional diversity in living organisms (Liu and Nes, 2009). Sterols that are present in the plasma membranes of eukaryotic cells are essential for the organisation and function of these structures, with ergosterol being the major component in lower eukaryotes, whereas cholesterol is found in higher eukaryotes (Hsueh *et al*., 2005; Xu *et al*., 2001), and sitosterol is the most abundant phytosterol (Roche *et al*., 2008). These steroids differ structurally, with ergosterol having two additional double bonds (at positions C7–C8 and C22–C23) and a methyl group at C24 of the side-chain (Fig. 2). Such features are essential for functionality and appear relatively late during ergosterol biosynthesis (Liu and Nes, 2009; Naumowicz *et al*., 2011). These structural differences also presumably allow the plant cell to recognise ergosterol as a 'non-self', foreign MAMP (Fig. 3).

Unfortunately, the mechanism of ergosterol recognition by plant cells is not known thus far. Scientists have hypothesised that plants either possess an ergosterol receptor/exploit an alternative MAMP receptor, or that ergosterol uptake leads to perturbations of a lipid raft structure because of the ability of this sterol to form very stable microdomains (Amborabé *et al*., 2003; Xu *et al*., 2001). Although the signal transduction pathway induced after recognition has not been fully elucidated to date, researchers have shown that ergosterol acts as a MAMP molecule in tobacco and tomato plants, resulting in an MTI response.

SOLDIERS AT THE LOOK-OUT POSTS: PROTEINS INVOLVED IN PERCEPTION OF FUNGAL ATTACKERS

Structural constituents of fungal cell membranes and walls induce the same types of basal innate immunity as other well-studied elicitors, such as LPS and flagellin (Iriti and Faoro, 2007; Zipfel *et al*., 2004). Fungal MAMPs include ergosterol, β-glucans, chitin/chitosan, ethylene-inducing xylanase and the very recently reported cerato-platanin BcSpL1 from *Botrytis cinerea*, whereas secreted elicitin proteins, such as cryptogein, have been reported from oomycetes, such as *Phytophthora* (Amborabé *et al*., 2008; Frías *et al*., 2013; Shimizu *et al*., 2010; Zipfel, 2009).

The initiation of an immune response involves the binding of a MAMP or effector to either a transmembrane PRR or an intracellular receptor (Thomma *et al*., 2011), with the PRRs being classified into two classes, namely receptor-like kinases (RLKs) and receptor-like proteins (RLPs) (Monaghan and Zipfel, 2012; Yang *et al*., 2012). To date, only a few plant PRRs for fungal MAMPs have been identified. The best-described include those that perceive chitin, namely chitin-elicited receptor kinase 1 (CERK1) and chitin elicitor binding protein (CEBiP) (Zipfel, 2009), the extracellular domains of which consist of leucine-rich repeats (LRRs) and are more specifically denoted as eLRRs (Mazzotta and Kemmerling, 2011; Yang *et al*., 2012). The CERK1/CEBiP receptor's activity and interaction with chitin have been shown *in vitro*, and seem to be responsible for conferring some resistance to fungi in plants. Furthermore, knockouts of *OsCERK1* in rice consequently reduced the capacity of the plant to perceive and adequately defend itself against fungal pathogens (Shimizu *et al*., 2010; Yang *et al*., 2012), whereas a very recent communication reported on

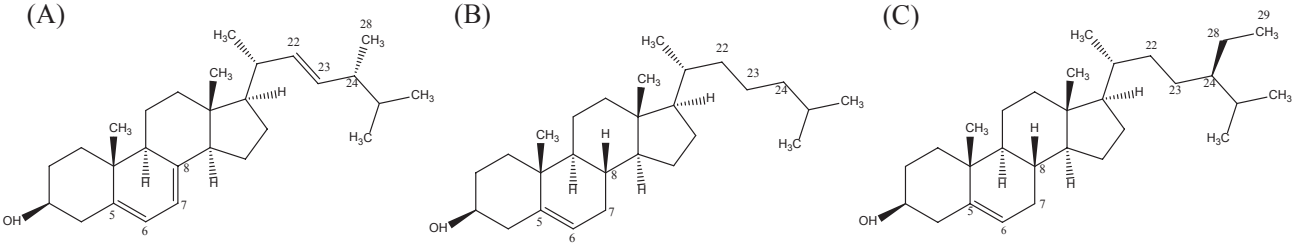


Fig. 2 Chemical structures of ergosterol (A), cholesterol (B) and sitosterol (C), indicating structural similarities and differences. Compared with cholesterol, ergosterol has unsaturation at positions C7–C8 and an additional methyl group (C28) at the C24 position, whereas sitosterol has an additional ethyl group (C28–C29) at the C24 position.

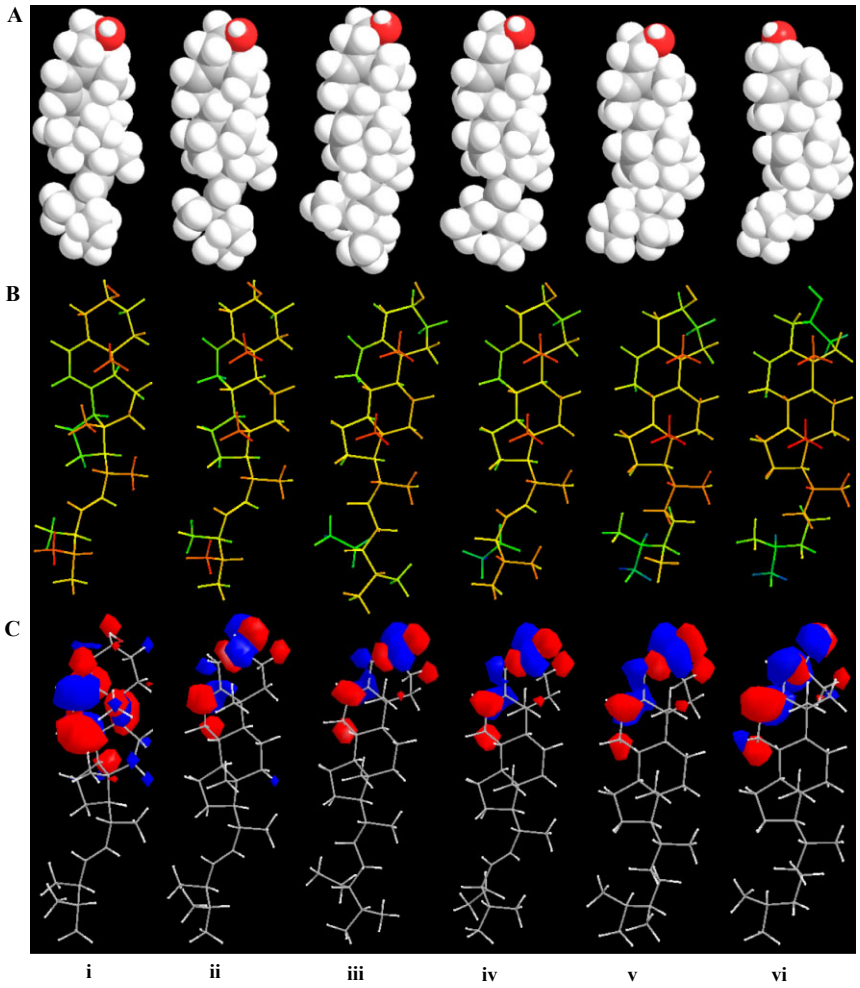


Fig. 3 Spacefilling (A), stereochemical stick (B) and frontier molecular orbital (FMO) (C) models for ergosterol (i), brassicasterol (ii), sitosterol (iii), stigmasterol (iv), campesterol (v) and cholesterol (vi). In (A), lone electron pairs (white circles) are indicated on the OH (red) groups of all sterols. In (B), the depth of colour indicates the progression of stereochemistry as planar (yellow/orange), forward (orange/red) and backward (green/blue) projections. In (C), the FMO surfaces (colour-coded wavefunctions: red, positive; blue, negative) visually represent the various stable electron distributions of a molecule and, according to frontier orbital theory, the shapes and symmetries are crucial in predicting the reactivity of a species, and the stereochemical and regiochemical outcome of a chemical reaction.

CERK1-independent fungal resistance in *Arabidopsis* (Narusaka *et al*., 2013). Further research is, however, required to definitively establish the roles played by these two important receptors in fungal pathogen perception and defence with regard to chitin and various chito-oligosaccharides. The ethylene-induced 22-kDa xylanase protein from *Trichoderma viride*, however, has been shown to be a prominent fungal elicitor, and has been demonstrated to stimulate ethylene production during the defence response. Two PPRs in tomato cells have also been identified, and fall into the LRR-RLP class of PRRs, namely LeEIX1 and LeEIX2. Only LeEIX2 is capable of inducing a defence response, whereas LeEIX1 assists LeEIX2 and aids endocytosis of the chitin-bound receptors (Mazzotta and Kemmerling, 2011).

SOUNDING THE ALARM: TRANSDUCTION OF ERGOSTEROL-TRIGGERED SIGNALS

On binding of an elicitor to a receptor, a signalling cascade is activated and, in turn, results in the activation and expression of multiple defence-related genes, with the corresponding proteins translated from their mRNA counterparts being the key players in many signal transduction pathways that activate the initial defence responses when the plant perceives MAMPs (Sanabria *et al*., 2012).

Akin to most plant–fungus interactions, signal transduction events specifically set in motion following the perception of ergosterol are characterised by changes in plasma membrane potential (Amborabé *et al*., 2003; Rossard *et al*., 2006), changes in the fluxes of H^+ ions (Amborabé *et al*., 2003; Granado *et al*., 1995; Kasparovsky *et al*., 2003; Rossard *et al*., 2010), rapid transient growth medium alkalinisation (Amborabé *et al*., 2003; Granado *et al*., 1995), mobilisation of internal calcium stores, activation of an oxidative burst and production of ROS (Kasparovsky *et al*., 2003), involving protein kinases and phospholipases, namely protein kinase C (PKC) and phospholipase A_2 (PLA_2) (Kasparovsky *et al*., 2004).

Regulatory enzymes are an essential part of these signal transduction pathways, and the most prominent participants are protein kinases and phosphatases, which act in concert to activate and deactivate, respectively, protein kinase components in signal

transduction during plant–pathogen interactions (Gerber and Dubery, 2004; Gerber *et al*., 2006; Xing *et al*., 2002; Zhu and Li, 2013). Furthermore, these cascades have been found to be a converging point at the start of the defence-signalling network (Angelova *et al*., 2006; He *et al*., 2007; Peck *et al*., 2001; Zhang and Klessig, 2001).

ENTER THE OMICS IN PLANT–FUNGUS INTERACTIONS FOR SETTING THE CROSSHAIRS ON ERGOSTEROL

The outcome of plant–fungus interactions relies on the complex interplay of numerous molecular signals, and both parties have elaborate pathways employed specifically in perception, recognition and infection/defence mechanisms. As defence and immune mechanisms against fungal MAMPs and, in particular, ergosterol are poorly understood relative to their bacterial counterparts, the challenge in current research is to use modern '-omic' technologies to unravel and reveal the cellular events and signal transduction pathways that are activated in plants following the perception of fungal pathogens or MAMPs (Ferreira *et al*., 2006).

Although numerous plant defence responses to fungal pathogens have already been studied and characterised, these have, to a large extent, been rather narrowly focused, and more recent strategies seem to favour bio-analytical techniques as well as more complete, comprehensive experimental designs. For this reason, it is important to consider all-inclusive research approaches that focus on '-omics' techniques, including genomics, transcriptomics, proteomics and metabolomics, thereby providing a greater wealth of potential knowledge that can be gained from a particular investigation (Lederberg and McCray, 2001).

Activating the defensome: transcription factors (TFs) and regulatory proteins

The activation and increased production of specific enzymes, hormones and metabolites in the host following pathogen perception are regarded as vital components of the ensuing host response. However, the importance of assessing gene regulation following host invasion and pathogenic challenge should not be neglected in order to more completely verify and evaluate the interplay between the two parties in such complex immune response cascades. As such, a large number of genes have also been identified as possible candidates that code for key proteins involved in a wide variety of plant–pathogen interactions. Many of these genes have been positively identified as being involved either directly or indirectly in the pathogen perception of plants.

To date, numerous studies have focused specifically on genomic/transcriptomic findings and, in particular, on TFs and gene regulatory elements that play commanding roles in transcriptional reprogramming of the host cell transcriptome, thereby initiating and potentiating the increased expression of defence biomolecules. It seems that some of these early defence responses are mediated by the expression of numerous WRKY TFs, which contain a domain having a highly conserved WRKYGQK sequence, as well as a novel zinc finger motif (Eulgem *et al*., 2000). These TFs have been shown to be involved in plant–fungus interactions. For example, overexpression of *AtWRKY80* in *Arabidopsis* leads to the activation of numerous defence responses, whereas *AtWRKY8* and *AtWRKY70* have been shown to control cross-talk between jasmonic acid (JA)- and salicylic acid (SA)-dependent defence response pathways and exhibit increased resistance to *Botrytis* infection (Ishihama *et al*., 2011). Additional investigations on *WRKY70* knockout lines of *Arabidopsis* have also shown increased susceptibility to infection by *B. cinerea*, thereby indicating the importance of this class of TFs in plant–fungus defences (Chen *et al*., 2010; Knoth *et al*., 2007; Li *et al*., 2006).

Furthermore, in a recent study conducted by Peng *et al*. (2012), it was found that the *OsWRKY30* gene was involved in specific defence responses in rice (*Oryza sativa*) following various treatments, including treatment of the host with JA and methyl jasmonate, as well as SA and the fungal rice pathogens, *Rhizoctonia solani* and *Magnaporthe grisea*. *OsWRKY30* showed increased transcript accumulation post-JA and SA treatment, and overexpression of this gene also led to the activation of numerous defence genes involved in the JA synthesis and defence-signalling pathways, including *LOX* (lipoxygenase), *AOS2* (allene oxide synthase 2), *PR-3* and *PR-10*, and increased JA accumulation following fungal pathogen infection.

Research conducted on *AtWRKY62* has also found a link between its activated expression through both SA- and JA-dependent signalling, with NPR-1 (Nonexpressor of pathogenesis-related gene 1) being involved in its activity (Pandey and Somssich, 2009). Unfortunately, these putative pathways have not yet been well studied, and *AtWRKY62* involvement in general immune responses is not clearly understood. Yet, other studies have shown the involvement of closely related WRKYs 18, 40 and 60 in *Arabidopsis* plant resistance to various pathogens. These three WRKYs seem to be interlinked, and may provide functional redundancy in both fungal and bacterial defence responses (Pandey and Somssich, 2009). *AtWRKY33* appears to be involved in defence against necrotrophic pathogens, and its putative interactions with *WRKY3* and *WRKY4* genes, and with *WRKY48*, suggest functional redundancy regarding these responses. Interactions of some of the more common, well-studied WRKY genes are summarised in Fig. 4 with regard to fungal MAMP perception in plants. It is clear that our understanding of the interconnected responses of individual members of the complex plant WRKY network is still not complete, but the large amount of research currently being conducted on this class of TFs is sure to provide further insights into plant defence responses in the future (Pandey and Somssich, 2009).

Key transcripts that have proven to be useful in the study of plant responses towards ergosterol are the type-I lipid-transfer proteins (LTPs). In a study conducted in 2006 on grapevine, *Vitis vinifera*, it was found that *VvLTP1* exhibited activated expression following ergosterol treatment and, subsequent to intensive studies of the gene's promoter region, several *cis*-acting regulatory elements were identified that may be involved in general and ergosterol-specific plant defence response activation of LTP genes (Laquitaine *et al*., 2006). The various regulatory elements identified within the promoter region of the gene were tested for activation by deletional analysis of the promoter region linked to β-glucuronidase (GUS) as a reporter gene (Laquitaine *et al*., 2006). Following ergosterol elicitation, it was postulated that one W-Box, EIRE (ethylene-induced response element), as well as a further identified W-Box, may be required for *VvLTP1* gene activation initiated specifically by ergosterol (Laquitaine *et al*., 2006). Furthermore, expression of *VvMYB4* and *VvWRKY1* genes coding for TFs associated specifically with binding to W-Box and MYB-box *cis*-acting promoter elements (Stracke *et al*., 2001; Ülker and Somssich, 2004) increased the expression of *VvLTP1* to greater than 10-fold *in vitro*, whereas *VvMYB2* increased in expression by approximately eightfold. In addition, following ergosterol elicitation, a 23-fold increase in the expression of *VvWRKY1* was noted within 5 h post-treatment, and a near fivefold increase in *VvLTP1* expression within 24 h post-treatment, specifically, thus highlighting the involvement of all the above in defence-related *VvLTP1* activation initiated specifically by ergosterol (Laquitaine *et al*., 2006). Moreover, it was found that the priming of grapevine with ergosterol resulted in enhanced resistance to *B. cinerea* and the up-regulated expression of *VST1* (coding for the defence-related enzyme stilbene synthase). This gene is responsible for increased phytoalexin production and plays an active role in defence against stilbene-sensitive pathogens (Coutos-Thévenot *et al*., 2001), and was also discovered, together with the accumulation of the phytoalexin resveratrol, following ergosterol elicitation (Laquitaine *et al*., 2006).

Positioning the infantry: the PR proteins

In addition to the elucidation of early plant defence responses, some studies have also been conducted on ergosterol's involvement in the activation of specific defence genes and thus subsequent protein counterparts. It has been well documented that a large arsenal of defence-related genes is expressed and the most prominent are the *PR* genes, responsible for the production of PR proteins (Van Loon *et al*., 2006). PR proteins are one of the most important aspects of immune response mechanisms and have been grouped into 17 established families (Naseri *et al*., 2012; Sels *et al*., 2008), and have been classified according to structure, function and biological activity (refer to Table 1). It should be noted that Table 1 only summarises the main PR protein family groups and their antifungal functions, but there are also subfamilies that are distinguished by particular properties. In addition, not all of the PR protein families have been identified for one individual plant species and, in some cases, are not present at all. As such, a brief description of the individual PR proteins identified specifically in ergosterol responses is given.

One study in particular focused on specific expressional changes and accumulation of transcripts of a number of genes coding for PR proteins, which may be distinctively grouped according to their physical and chemical properties, cellular localisation and purported functions in plant defence responses in tobacco following ergosterol elicitation (Lochman and Mikes, 2006). On investigation of the specific defence mechanisms following ergosterol perception, it was found that PR-1, PR-3 and PR-5 were expressed (Lochman and Mikes, 2006), as well as the PR-14 [non-specific (ns)LTPs] family (Laquitaine *et al*., 2006), in accordance with increased gene transcript levels.

Five separate PR protein coding genes (*PR1a*, *PR1b*, $PR1_{basic}$, *PR3Q* and *PR5*), together with the genes *PI-I* and *PI-II* coding for proteinase inhibitors, were also investigated in *Nicotiana tabacum* following elicitation with nanomolar concentrations of ergosterol. It was found that transcripts of both *PR1a* and *PR1b* rapidly accumulated and relative expression increased to greater than 60- and 10-fold, respectively, for these candidates over a period of 48 h following ergosterol infiltration. Furthermore, transcript levels of both *PR3Q* and *PR5* genes also showed increases in relative gene expression of greater than fourfold over a 24-h period, with *PR3Q* expression increasing to more than 10-fold within 48 h post-elicitation (Lochman and Mikes, 2006).

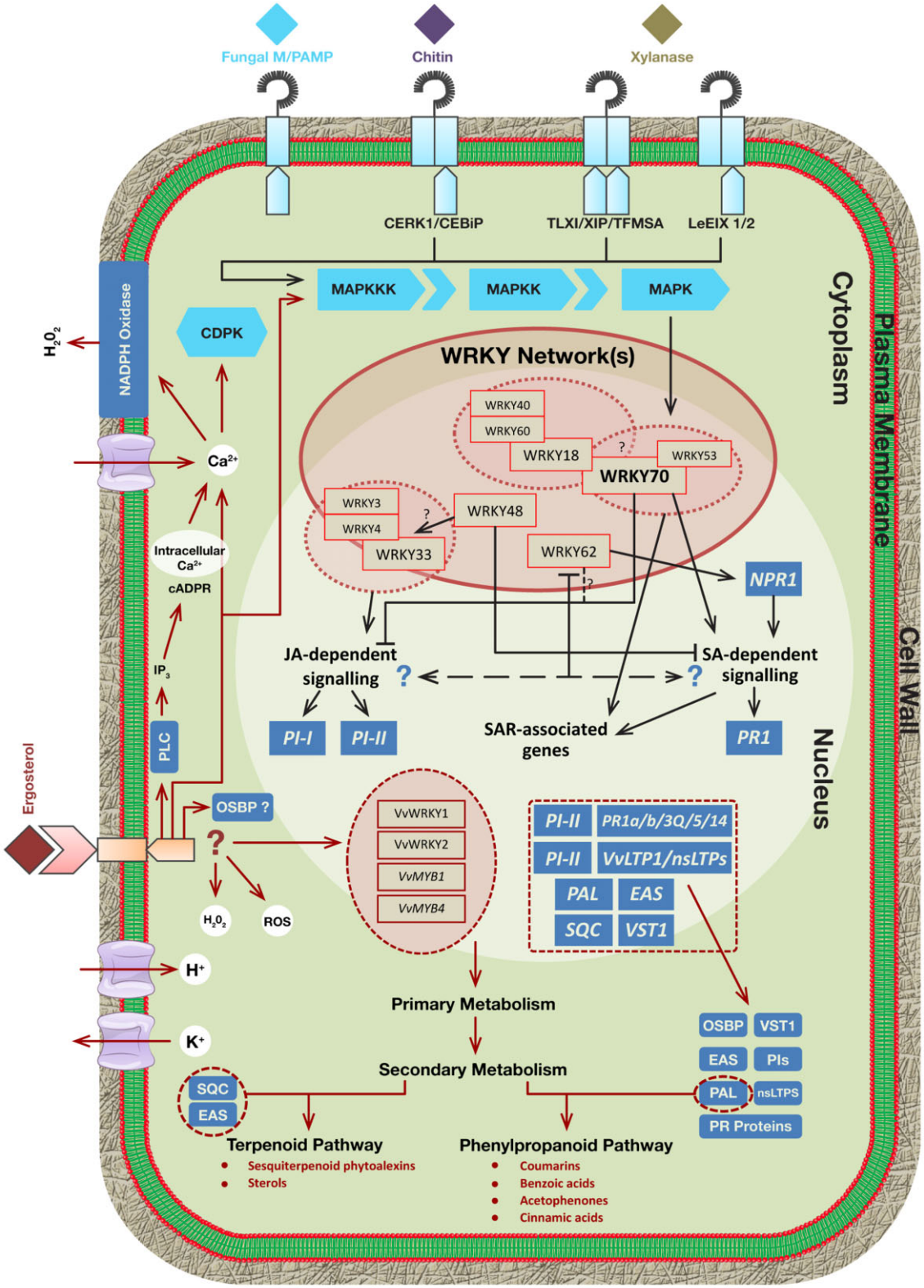
It was interesting to note that transient increases in relative expression of *PI-I* and *PI-II* genes were observed following leaf infiltration with three separate sterols (cholesterol, sitosterol and stigmasterol) employed as controls, as well as with water, with maximum expression occurring at 24 h post-elicitation. This was expected as the up-regulated expression of proteinase inhibitors has been strongly linked to plant wounding, stress responses and JA application among others, as would be evoked through leaf infiltration (Choi *et al*., 2000; Linthorst *et al*., 1993). However, this transient increase in the relative expression of *PI-I* and *PI-II* genes was not seen following ergosterol infiltration, thereby suggesting a potential suppression of these genes and thus the potential downstream pathways that may be affected (Lochman and Mikes, 2006). From all the analysed relative expression changes in the genes investigated, it was thus postulated that ergosterol may function to increase the expression of certain key defence-related genes, specifically those involved in or affected by the SA signalling pathway, and that ergosterol may mediate possible cross-talk between SA and JA signalling pathways, leading to the inactivation of the JA signalling pathway in tobacco (Lochman and Mikes, 2006).

Fig. 4 Summary of all known/putative mechanisms and players of important fungal microbe/pathogen-associated molecular pattern (M/PAMP) perception, and those influenced by ergosterol. The structures of the known fungal pattern recognition receptor (PRR) complexes (those representing the recognition of chitin and xylanase) have been included and indicate downstream/intracellular mechanisms activated by their perception at the cell membrane. These mechanisms can be extended to provide a suitable hypothesis for ergosterol perception. Ergosterol has been shown to trigger both Ca^{2+} influx and release of intracellular Ca^{2+} stores, which has been observed with the perception of other fungal M/PAMPs by the plant immune system. The hypothesised receptor, based on the assumption that ergosterol may be perceived in the same manner as other M/PAMPs, is indicated in maroon on the left. A possible intracellular receptor, oxysterol-binding protein (OSBP), is included. The known metabolic pathways, and their resultant metabolites, that are involved in the defence response following ergosterol treatment are shown (maroon arrows). In addition, putative interactions between some well-studied WRKY transcription factors are also represented within the nucleus, creating an intricate WRKY network activated through various upstream interactions by MAPK-based signalling. Dotted lines indicate putative/unestablished reactions, and question marks represent unknown mechanisms of interaction. Three important metabolic enzymes, which contribute towards the production of specific ergosterol-induced metabolites, are SQC, EAS and PAL. These are circled by dotted lines in the lower portion of the figure. It is important to note that the genes, proteins and metabolites implicated in this figure have resulted from studies in tobacco and grape plants. cADPR, cyclic ADP-ribose; CDPK, calcium-dependent protein kinase; CeBiP, chitin elicitor binding protein; CERK1, chitin elicitor receptor kinase; EAS, *epi*-aristolochene synthase; H_2O_2, hydrogen peroxide; IP3, inositol 3-phosphate; JA, jasmonic acid; LeEIX, *Lycopersicon esculentum* ethylene-induced xylanase; MAPK, mitogen-activated protein kinase; MAPKK, MAP kinase kinase; MAPKKK, MAP kinase kinase kinase; NPR1, Nonexpressor of pathogenesis-related gene 1; nsLTP, nonspecific lipid-transfer protein; MYB, myeloblastosis; OSBP, oxysterol-binding protein; PAL, phenylalanine ammonia lyase; PLC, phospholipase C; PI, proteinase inhibitor; PR, pathogenesis-related; ROS, reactive oxygen species; SA, salicylic acid; SAR, systemic acquired resistance; SQC, sesquiterpenoid cyclase; TFs, transcription factors; TFMSA, trifluoromethanesulphonic acid; TLXI, thaumatin-like xylanase inhibitor; VST1, *Vitis* stilbene synthase 1; Vv, *Vitis vinifera*; XIP, xylanase inhibitor protein.

Table 1 Summary of classified pathogenesis-related (PR) proteins in plant systems, and their general antimicrobial and antifungal activities.

Family	Original discovery	Common name	Function	References
PR-1	Tobacco PR-1a	PR-1	Unknown, possible antifungal activity	Asensio *et al*. (2004); Elvira *et al*. (2008); Zhu *et al*. (2012)
PR-2	Tobacco PR-2	β-1,3-Glucanases	Cell wall degradation	Ebrahim *et al*. (2011); Vigers *et al*. (1991)
PR-3	Tobacco P, Q	Chitinases	Enzymatic degradation of chitin fragments in fungal cell walls	Ebrahim *et al*. (2011); Sharma *et al*. (2011); Torres *et al*. (2012)
PR-4	Tobacco 'R'	Chitin-binding proteins	Assist with chitin hydrolysis by chitinase	Borad and Sriram (2008); Li *et al*. (2010)
PR-5	Tobacco S	Thaumatin-like proteins (TLPs), permatin, osmotin	Permeabilise fungal and other cell membranes, inhibit hyphal growth	Borad and Sriram (2008); Grenier and Asselin (1990); Naseri *et al*. (2012); Van Loon and Van Strien (1999)
PR-6	Tomato inhibitor I	Proteinase inhibitors	Inhibition of fungal enzymes, prevent chitin synthesis	Van Loon and Van Strien (1999)
PR-7	Tomato P_{69}	Endoproteinases	Endoproteinase activity	Van Loon *et al*. (2006)
PR-8	Cucumber chitinase	Class III endochitinases, glycosyl hydrolases	Lysozyme activity	Edreva (2005); Van Loon and Van Strien (1999); Wanderley-Nogueira *et al*. (2012)
PR-9	Tobacco	Peroxidases	Involved in enzymatic cross-linking of cell wall components	Edreva (2005); Li *et al*. (2010)
PR-10	Parsley 'PR1'	Ribonucleases/ribosome inactivating protein	Enzymatic cleavage of viral RNA	Borad and Sriram (2008); Li *et al*. (2010); Soh *et al*. (2011)
PR-11	Tobacco class V chitinase	Chitinases	Fungal cell wall degradation	Edreva (2005)
PR-12	Radish Rs-AFP3	Lipid-transfer protein (LTP), defensins	Transfer phospholipids between membranes, fungal membrane permeabilisation	Kader (1996); Sels *et al*. (2008)
PR-13	*Arabidopsis* THI2.1	Thionins	Permeabilise cell membranes	Sels *et al*. (2008); Van Loon and Van Strien (1999)
PR-14	Barley LTP4	LTPs, nonspecific (ns)LTPs	Involved in cutin synthesis Transport of sterol molecules across cell membranes and possible ergosterol binding (nsLTPs in rice)	Blilou *et al*. (2000); Cheng *et al*. (2004); Van Loon and Van Strien (1999)
PR-15	Barley OxOa	Oxalate oxidases, germins	Unknown	Bernier and Berna (2001); Zhang *et al*. (1995); Zhou *et al*. (1998)
PR-16	Barley OxOLP	Germin-like proteins	Unknown	Van Loon *et al*. (2006)
PR-17	Tobacco PRp27	Basic secretory proteins	Unknown	Van Loon *et al*. (2006)

nsLTPs are small, basic, cysteine-rich proteins whose function in plants has been a matter of intense debate since their initial discovery in the 1970s (Kader, 1975). Potential roles have been postulated as to their involvement in intracellular phospholipid trafficking (Kader, 1975), somatic embryogenesis and plant developmental mechanisms (Coutos-Thévenot *et al*., 1993; Fleming *et al*., 1992), transport of structural molecules, including cutin monomers, in plants during cuticle assembly (Meijer *et al*., 1993; Sterk *et al*., 1991) and, more recently, putative roles in numerous plant defence responses (Blilou *et al*., 2000; Carvalho *et al*., 2001).


Fungal M/PAMP
Chitin
Xylanase
CERK1/CEBiP
TLXI/XIP/TFMSA
LeEIX 1/2
MAPKKK
MAPKK
MAPK
Cytoplasm
Plasma Membrane
Cell Wall
Nucleus
NADPH Oxidase
H_2O_2
CDPK
Ca^{2+}
Intracellular Ca^{2+}
cADPR
IP_3
PLC
Ergosterol
OSBP ?
?
H_2O_2
ROS
H^+
K^+
WRKY Network(s)
WRKY40
WRKY60
WRKY18
WRKY53
WRKY70
WRKY3
WRKY4
WRKY33
WRKY48
WRKY62
NPR1
JA-dependent signalling
SA-dependent signalling
PI-I
PI-II
SAR-associated genes
PR1
VvWRKY1
VvWRKY2
VvMYB1
VvMYB4
PI-II
PR1a/b/3Q/5/14
PI-II
VvLTP1/nsLTPs
PAL
EAS
SQC
VST1
Primary Metabolism
Secondary Metabolism
SQC
EAS
Terpenoid Pathway
Sesquiterpenoid phytoalexins
Sterols
Phenylpropanoid Pathway
Coumarins
Benzoic acids
Acetophenones
Cinnamic acids
OSBP
VST1
EAS
PIs
PAL
nsLTPS
PR Proteins

In a 2003 study utilising grape cell suspensions, the changes in expression of certain *PR-14* genes coding for several nsLTPs were studied in response to various elicitor molecules, including both ergosterol and a protein fraction purified from *B. cinerea*. Three full-length nsLTP cDNA clones were initially identified in two grape cultivars and the full-length cDNA clone of *41B-nsLTP* was subsequently employed as a probe for Northern blot analyses of *PR-14* (nsLTP) gene expression status in the cell cultures following elicitor induction (Gomès *et al*., 2003). Ergosterol and the uncharacterised purified *B. cinerea* protein fraction drastically increased the hybridisation signals following elicitor induction, but only minor expression increases were noted following treatment with cholesterol and sitosterol. Moreover, SA had a negligible effect on gene expression status, whereas JA showed a small but significant increase in gene induction (Gomès *et al*., 2003). To further assess the expression kinetics in response to ergosterol and the *B. cinera* fraction, these two elicitors were studied on cell suspensions, and it was found that nsLTP transcripts increased at 5 h post-ergosterol treatment (150-fold) and 10 h post-*B. cinerea* proteinaceous elicitor treatment (190-fold) (Gomès *et al*., 2003).

Deploying the chemical defence units: the metabolomic squadrons

In order to deal with a large number of biotic and abiotic stressors, plants have evolutionarily developed a metabolic network that is highly diverse and complex when compared with that of other organisms. The main challenge of metabolomics studies, however, is the enormous complexity and diversity of the metabolome (Allwood *et al*., 2010; Dunn and Ellis, 2005; Sumner *et al*., 2003), and it should be noted that the application of metabolomics to the investigation of plant–pathogen interactions is still in its infancy (Okazaki and Saito, 2012; Tugizimana *et al*., 2013).

The chemical defence arsenal of plants can include pre-formed antimicrobial phytoanticipins, described as 'low-molecular-weight' antimicrobial compounds that are present in plants before challenge by microorganisms, or are produced after infection solely from pre-existing precursors (Bourgaud *et al*., 2001; Dixon, 2001; Essenberg, 2001; Grayer and Kokubun, 2001; Mert-Türk, 2002). In contrast, phytoalexins are host-synthesised, low-molecular-weight compounds with protective properties (antimicrobial, photoprotective, structure stabilising and signalling), the *de novo* biosynthesis and accumulation of which are induced in plants following biotic or abiotic stress/attack, i.e. the production of phytoalexins requires the *de novo* activation and expression of the enzymes involved in their respective biosynthetic pathways (Edreva *et al*., 2008; Mert-Türk, 2002; Pedras and Ahiahonu, 2005). Phytoalexins include a wide spectrum of compounds that form structurally and functionally diverse metabolite families, such as stilbenes, coumarins, sesquiterpenes and polyketides, as well as other phenylpropanoid and flavonoid derivatives (Hammerschmidt, 1999; Smith, 1996). More than 300 phytoalexins have now been characterised from the members of more than 20 families. Generally, plants of a particular family produce phytoalexins that are structurally related (Dixon, 2001; Harborne, 2000).

Various studies have reported the antifungal activity of phytoalexins against numerous phytopathogenic fungi, also revealing a complex relationship between phytoalexin accumulation and disease resistance that is greatly pathogen dependent (Ahuja *et al*., 2012; Mert-Türk, 2002; Tierens *et al*., 2001). The work of Huffaker *et al*. (2011) describes maize-produced acidic sesquiterpenoid phytoalexins, zealexins, which significantly inhibited the growth of fungal pathogens at physiologically relevant conditions. Furthermore, deficiency in phytoalexin production has been shown to enhance the susceptibility of plants to phytopathogenic fungal infection (Glazebrook *et al*., 1997; Thomma *et al*., 1999). However, in their continuous adaptation and co-evolution with plants, some phytopathogenic fungi have acquired efficient abilities to overcome host chemical defences through enzyme-mediated detoxification of phytoalexins and phytoanticipins (Pedras and Ahiahonu, 2005; Schmidt and Panstruga, 2011). It has been shown, for instance, that the phytopathogenic fungus, *Gibberella pulicaris*, a major cause of dry-rot of stored potatoes (*Solanum tuberosum*), has the ability to detoxify the sesquiterpene phytoalexins, rishitin and lubimin, produced by potato by enzyme-mediated epoxidation, dehydrogenation and cyclisation (Desjardins *et al*., 1992; Gardner *et al*., 1994; Pedras and Ahiahonu, 2005).

The perception of ergosterol results in the activation of a number of defence genes and the production of defence-related secondary metabolites (Kasparovsky *et al*., 2003, 2004; Lochman and Mikes, 2006). Lochman and Mikes (2006) also demonstrated that the treatment of tobacco cells with nanomolar concentrations of ergosterol led to the *de novo* gene expression of *PAL* genes (coding for phenylalanine ammonia lyase, a key defence-related enzyme responsible for the initiation of the phenylpropanoid pathway that ultimately leads to the production of SA and other phenolics). Furthermore, 5-*epi*-aristolochene synthase and sesquiterpene synthase (important enzymes related to the synthesis of various sesquiterpenoid phytoalexins) were responsive towards ergosterol (Lochman and Mikes, 2006; Rossard *et al*., 2010).

Although it is known that ergosterol elicits the synthesis of phytoalexins, such as resveratrol (grapevine) and capsidiol (potato and tobacco) (Kasparovsky *et al*., 2003; Robert *et al*., 2001, 2002), the work of Tugizimana *et al*. (2012) is the only study reported to have investigated the effect of ergosterol on plant secondary metabolism from a metabolomic perspective. Five sesquiterpenoid phytoalexins (capsidiol, lubimin, phytuberin, rishitin and solavetivone) were identified/annotated, indicating ergosterol-induced activation of the terpenoid pathway. Moreover, the study

indicated that the perception of ergosterol, acting as a 'non-self' M/PAMP molecule, induces significant and dynamic metabolomic alterations in tobacco cell suspensions. These changes included variation in the levels of the constitutively expressed metabolites and the production of new metabolites (Tugizimana *et al*., 2014).

POTENTIAL ERGOSTEROL SENSOR PROTEINS AS HOST SENTINELS

Although modern approaches with regard to plant–fungus interactions have certainly yielded significant insights to date, no clear model pertaining to ergosterol perception and the subsequent signalling cascade has yet been established (Fig. 4).

Data presented by Vega and Boland (1988) first suggested the existence of binding sites in plants for sterols (endogenous sitosterol and stigmasterol), although both the biological role and specificity with respect to 'self' and 'non-self' recognition remained to be examined. Plant cells are presumably able to recognise ergosterol as 'non-self' (Sanabria *et al*., 2010) because of the distinguishing structural features of the molecule (Figs 2 and 3). Given that ergosterol has been considered as a MAMP molecule (Nürnberger *et al*., 2004), it would be rational to assume that there exists a receptor for perception. Granado *et al*. (1995) reported a stimulus–response perception system for ergosterol in tomato cells. Here, subnanomolar concentrations of ergosterol purified from *Cladosporium fulvum* spore extracts were found to be able to elicit extracellular alkalinisation with a selectivity and sensitivity that resembled steroid hormone perception in animals. None of the endogenous plant sterols that were evaluated (sitosterol, stigmasterol and campesterol) exhibited similar activity and, based on these observations, the authors postulated that plants possess ergosterol receptors for the detection of fungi in plant–pathogen or plant–symbiont interactions, thereby adding to the concept of receptor-mediated sterol perception. A similar postulation with hydrogen peroxide-associated signalling was made a year later by Kauss and Jeblick (1996).

Later studies confirmed specific ergosterol perception in comparative investigations with other sterols (sitosterol, stigmasterol, campesterol and cholesterol) by plants (Rossard *et al*., 2010; Vatsa *et al*., 2011a). The former noted that, if *Beta vulgaris* leaf cells were first exposed to ergosterol, a refractory state resulted which was characterised by a change in pH of the extracellular environment. Following a second application of ergosterol after a 2-h period, no secondary pH changes were observed. However, after a first application with one of the other sterols or chitosan, cells responded to a subsequent application of ergosterol. This is indicative that ergosterol perception does not interfere with that of the other tested sterols and chitosan, and vice versa, and could imply that plants possess a specific receptor for ergosterol. These results pointed to an ergosterol-sensitive recognition mechanism in *Beta vulgaris* leaf cells that shares many of the properties of well-described recognition systems for MAMPs, such as flg22, elf, chitin and LPS (Boller and Felix, 2009; Gerber and Dubery, 2004), including transient increases in $[Ca^{2+}]_{cyt}$, modification of proton fluxes, an oxidative burst and activation of MAPKs corresponding to SIPK and WIPK, which was also the case observed with cryptogein treatment of *N. tabacum* cells (Vatsa *et al*., 2011a). In the latter study pertaining to the structural analogues of ergosterol (cholesterol, sitosterol, stigmasterol and campesterol), only campesterol exhibited slight activity to trigger a rise in calcium concentration and a very low oxidative burst, suggesting that there are some shared but, more so, differential/specific structural features (Fig. 3) that are recognised by the perception system for ergosterol.

Osman *et al*. (2001) reported on the decrease in defence responses (Ca^{2+} signalling, extracellular alkalinisation and SAR) in tobacco following treatments with elicitin (cryptogein) mutated in its ability to bind sterols. From this study, it is very apparent that, in order for elicitins to trigger biological responses, the elicitors need to be sterol loaded. As oomycetes, such as *Phytophthora*, do not synthesise their own sterols, these would most likely be acquired from the plasma membranes of the host. Moreover, sterol loading of the elicitin was shown to be essential and a prerequisite for binding to high-affinity sites. As such, defence responses thus appear to be dependent on perception only if the elicitin is sterol loaded. Interestingly, however, Trigos *et al*. (2005) reported on the identification of ergosterol in *Phytophthora dreschsleri*, a soil-borne phytopathogen. The authors noted that this is an unusual metabolite for this oomycete member and is, to date, the only such documentation in this regard.

Research conducted by Avrova *et al*. (2004) showed the specific up-regulation of an oxysterol-binding protein-coding gene (*OSBP*) in potato following infection of plants with *Phytophthora infestans*. In mammalian systems, OSBP activity is divided between the endoplasmic reticulum and Golgi apparatus, where it imparts sterol-dependent regulation of phosphoinositide and sphingolipid pathways. It has been hypothesised further that OSBPs specifically bind to, and interact with, molecules such as ergosterol, and possibly play a role in vesicle trafficking in fungi and fungal perception in mammals and plants (Im *et al*., 2005). Furthermore, phytosterols may undergo oxidation and/or oxygenation in the presence of ROS (Burlini *et al*., 2011), thereby generating altered molecular patterns within the sterols (i.e. oxysterols) and ultimately resulting in defence responses. This, coupled with research conducted by Madala *et al*. (2012), which showed the specific up-regulation of an *OSBP* gene in *Arabidopsis thaliana* following LPS elicitation, makes OSBP an attractive target to study for specific pathways of fungal perception in plants, and the potential elucidation of convergent defence cascades employed in the recognition and defence against numerous phytopathogens.

Therefore, the potential/hypothesised perception mechanism(s) for ergosterol (Fig. 4) might involve a membrane-localised

receptor-like kinase(s) (analogous to the brassinosteroid receptor that binds steroid hormones; Hou *et al*., 2013; Yang *et al*., 2012) binding to/interacting with intracellular oxysterol-binding proteins, or localised perturbations of membrane structures [e.g. changes in the detergent-resistant microdomain (DRM) composition of lipid rafts and the activation of DRM-associated proteins, membrane depolarisation, opening of calcium channels, etc.]. In support of the latter, it has been reported that RLKs are over-represented in plant DRMs (Zappel and Panstruga, 2008), and the same has been reported for flagellin, cryptogein and chitin elicitation in plants (Cacas *et al*., 2012).

A BATTLE WON?

As part of their innate immune system, it would appear that plants have evolved a system for the recognition of exogenous sterols that probably targets the fungal-specific ergosterol. It is clear that, although modern approaches regarding plant–fungus interactions have certainly yielded significant insights to date, no clear model pertaining to ergosterol perception and the subsequent signalling cascades has yet been established. Further investigations of ergosterol as a MAMP, its recognition by plant cells and its ability to trigger MTI are still needed. The use of large-scale quantifiable tools, such as '-omics' approaches (e.g. genomics, transcriptomics, proteomics and metabolomics), will certainly provide comprehensive insights into the understanding of ergosterol–plant interactions. The discovery of a receptor for ergosterol would add impetus to this important area of research and generate renewed interest in its application in plant biotechnology and crop protection.

ACKNOWLEDGEMENTS

The South African National Research Foundation (NRF) and the University of Johannesburg are acknowledged for financial support.

REFERENCES

Ahuja, I., Kissen, R. and Bones, M.A. (2012) Phytoalexins in defense against pathogens. *Trends Plant Sci*. **17**, 73–90.

Allwood, J.W., Ellis, D. and Goodacre, R. (2008) Metabolomic technologies and their application to the study of plants and plant–host interactions. *Physiol. Plant*. **132**, 117–135.

Allwood, J.W., Clarke, A., Goodacre, R. and Mur, L.A.J. (2010) Dual metabolomics: a novel approach to understanding plant–pathogen interactions. *Phytochemistry*, **71**, 590–597.

Almagro, L., Gómez Ros, L.V., Belchi-Navarro, S., Bru, R., Ros Barceló, A. and Pedreño, M. (2009) Class III peroxidases in plant defence reactions. *J. Exp. Bot*. **60**, 377–390.

Amborabé, B.-E., Rossard, S., Pérault, J.M. and Roblin, G. (2003) Specific perception of ergosterol by plant cells. *C. R. Biol*. **326**, 363–370.

Amborabé, B.-E., Bonmort, J., Fleurat-Lessard, P. and Roblin, G. (2008) Early events induced by chitosan on plant cells. *J. Exp. Bot*. **59**, 2317–2324.

Angelova, Z., Georgiev, S. and Roos, W. (2006) Elicitation of plants. *Biotechnol. Biotechnol. Equip*. **20**, 72–83.

Arasimowicz, M. and Floryszakwieczorek, J. (2007) Nitric oxide as a bioactive signalling molecule in plant stress responses. *Plant Sci*. **172**, 876–887.

Asensio, T., Crespo, J.F., Sanchez-Monge, R., Lopez-Torrejon, G., Somoza, M.L., Rodriguez, J. and Salcedo, G. (2004) Novel plant pathogenesis-related protein family involved in food allergy. *J. Allergy Clin. Immunol*. **114**, 896–899.

Avrova, A.O., Taleb, N., Rokka, V.M., Heilbronn, J., Campbell, E., Hein, I., Gilroy, E.M., Cardle, L., Bradshaw, J.E., Stewart, H.E., Fakim, Y.J., Loake, G. and Birch, P.R.J. (2004) Potato oxysterol binding protein and cathepsin B are rapidly up-regulated in independent defence pathways that distinguish R-gene-mediated and field resistance to *Phytophthora infestans*. *Mol. Plant Pathol*. **5**, 45–56.

Beck, M., Heard, W., Mbengue, M. and Robatzek, S. (2012) The INs and OUTs of pattern recognition receptors at the cell surface. *Curr. Opin. Plant Biol*. **15**, 367–374.

Bent, A.F. and Mackey, D. (2007) Elicitors, effectors and R genes: the new paradigm and lifetime supply of questions. *Annu. Rev. Phytopathol*. **45**, 399–436.

Bernier, F. and Berna, A. (2001) Germins and germin-like proteins: plant do-all proteins. But what do they do exactly? *Plant Physiol. Biochem*. **39**, 545–554.

Bittel, P. and Robatzek, S. (2007) Microbe-associated molecular patterns (MAMPs) probe plant immunity. *Curr. Opin. Plant Biol*. **10**, 335–341.

Blilou, I., Ocampo, J.A. and Garcia-Garrido, J.M. (2000) Induction of LTP (Lipid transfer protein) and PAL (Phenylalanine ammonia-lyase) gene expression in rice roots colonised by the arbuscular mycorrhizal fungus *Glomus mosseae*. *J. Exp. Bot*. **51**, 1969–1977.

Boller, T. and Felix, G. (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern recognition receptors. *Annu. Rev. Plant Biol*. **60**, 379–406.

Borad, V. and Sriram, S. (2008) Pathogenesis-related proteins for the plant protection. *Asian J. Exp. Sci*. **22**, 189–196.

Bourgaud, F., Gravot, A., Milesi, S. and Gontier, E. (2001) Production of plant secondary metabolites: a historical perspective. *Plant Sci*. **161**, 839–851.

Burlini, N., Iriti, M., Daghetti, A., Faoro, F., Ruggiero, A. and Bernasconi, S. (2011) Benzothiadiazole (BTH) activates sterol pathway and affects vitamin D_3 metabolism in *Solanum malacoxylon* cell cultures. *Plant Cell Rep*. **30**, 2131–2141.

Cacas, J.-L., Furt, F., Le Guédard, M., Schmitter, J.-M., Buré, C., Gerbeau-Pissot, P., Moreau, P., Bessoule, J.-J., Simon-Plas, F. and Mongrand, S. (2012) Lipids of plant membrane rafts. *Prog. Lipid Res*. **51**, 272–299.

Carvalho, A.O., Machado, O.L.T., Cunha, M.A., Santos, I.S. and Gomes, V.M. (2001) Antimicrobial peptides and immunolocalisation of an LTP in *Vigna unguiculata* seeds. *Plant Physiol. Biochem*. **39**, 137–146.

Chen, L., Hamada, S., Fujiwara, M., Zhu, T., Thao, N.P., Wong, H.L., Krishna, P., Ueda, T., Kaku, H., Shibuya, N., Kawasaki, T. and Shimamoto, K. (2010) The Hop/Sti1-Hsp90 chaperone complex facilitates the maturation and transport of a PAMP receptor in rice innate immunity. *Cell Host Microbe* **7**, 185–196.

Cheng, C.S., Samuel, D., Liu, Y.J., Shyu, J.C., Lai, S.M., Lin, K.F. and Lyu, P.C. (2004) Binding mechanism of nonspecific lipid transfer proteins and their role in plant defence. *Biochemistry*, **43**, 13 628–13 636.

Choi, D., Park, J.A., Seo, Y.S., Chun, Y.J. and Kim, W.T. (2000) Structure and stress-related expression of two cDNAs encoding proteinase inhibitor II of *Nicotiana glutinosa* L. *Biochim. Biophys. Acta*, **1492**, 211–215.

Coutos-Thévenot, P., Jouenne, T., Maës, O., Guerbette, F., Grosbois, M., Le Caer, J.P., Boulay, M., Deloire, A., Kader, J.C. and Guern, J. (1993) Four 9-kDa proteins excreted by somatic embryos of grapevine are isoforms of lipid-transfer proteins. *Eur. J. Biochem*. **217**, 885–889.

Coutos-Thévenot, P., Poinssot, B., Bonomelli, A., Yean, H., Breda, C., Buffard, D., Esnault, R., Hain, R. and Boulay, M. (2001) *In vitro* tolerance to *Botrytis cinerea* of grapevine 41B rootstock in transgenic plants expressing the stilbene synthase Vst1 gene under the control of a pathogen-inducible PR-10 promoter. *J. Exp. Bot*. **52**, 901–910.

Deepak, S.A., Ishii, H. and Park, P. (2006) Acibenzolar-S-methyl primes cell wall strengthening genes and reactive oxygen species forming/scavenging enzymes in cucumber after fungal pathogen attack. *Physiol. Mol. Plant Pathol*. **69**, 52–61.

Desjardins, E.A., Gardener, W.H. and Weltring, M.K. (1992) Detoxification of sesquiterpene phytoalexins by *Gibberella pulicaris* (*Fusarium sambucinum*) and its importance for virulence on potato tubers. *J. Ind. Microbiol*. **9**, 201–211.

Dixon, R.A. (2001) Natural products and plant disease resistance. *Nature*, **411**, 843–847.

Dohnal, V., Jezkova, A., Pavlikova, L., Musilek, K., Jun, D. and Kuca, K. (2010) Fluctuation in the ergosterol and deoxynivalenol content in barley and malt during malting process. *Anal. Bioanal. Chem*. **397**, 109–114.

Dunn, W.B. and Ellis, D.I. (2005) Metabolomics: current analytical platforms and methodologies. *Trends Anal. Chem*. **24**, 285–294.

Ebrahim, S., Usha, K. and Singh, B. (2011) Pathogenesis-related (PR) proteins?: chitinase and β-1,3-glucanase in defence mechanism against malformation in mango (*Mangifera indica* L). *Sci. Hort*. **130**, 847–852.

Edreva, A. (2005) Pathogenesis-related proteins?: research progress in the last 15 years. *Gen. Appl. Plant Physiol.* **31**, 105–124.

Edreva, A., Velikova, V., Tsonev, T., Dagnon, S., Gürel, A., Aktaş, L. and Gesheva, E. (2008) Stress-protective role of secondary metabolites: diversity of functions and mechanisms. *Gen. Appl. Plant Physiol.* **34**, 67–78.

Elvira, M.I., Galdeano, M.M., Gilardi, P., García-Luque, I. and Serra, M.T. (2008) Proteomic analysis of pathogenesis-related proteins (PRs) induced by compatible and incompatible interactions of pepper mild mottle virus (PMMoV) in *Capsicum chinense* L3 plants. *J. Exp. Bot.* **59**, 1253–1265.

Essenberg, M. (2001) Prospects for strengthening plant defences through phytoalexin engineering. *Physiol. Mol. Plant Pathol.* **59**, 71–81.

Eulgem, T., Rushton, P.J., Robatzek, S. and Somssich, I.E. (2000) The WRKY superfamily of plant transcription factors. *Trends Plant Sci.* **5**, 199–206.

Fan, J. and Doerner, P. (2012) Genetic and molecular basis of nonhost disease resistance: complex, yes; silver bullet, no. *Curr. Opin. Plant Biol.* **15**, 1–7.

Ferreira, R.B., Monteiro, S., Freitas, R., Santos, C.N., Chen, Z., Batista, L.M., Duarte, J., Borges, A. and Teixeira, A.R. (2006) Fungal pathogens: the battle for plant infection. *Crit. Rev. Plant Sci.* **25**, 505–524.

Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L. and Gurr, S.J. (2012) Emerging fungal threats to animal, plant and ecosystem health. *Nature*, **484**, 186–194.

Fleming, A.J., Mandel, T., Hoffman, S., Sterk, P., De Vries, S.C. and Kuhlemeier, C. (1992) Expression pattern of a putative lipid transfer protein gene within the shoot apex. *Plant J.* **2**, 855–862.

Flor, H.H. (1942) Inheritance of pathogenicity in *Melampsora lini*. *Phytopathology*, **32**, 653–669.

Frías, M., Brito, N. and Gonzáles, C. (2013) The *Botrytis cinerea* cerato-platanin BcSpl1 is a potent inducer of systemic acquired resistance (SAR) in tobacco and generates a wave of salicylic acid expanding from the site of application. *Mol. Plant Pathol.* **14**, 191–196.

Gardner, W.H., Desjardins, E.A., McCormick, P.S. and Weisleder, D. (1994) Detoxification of the potato phytoalexin rishitin by *Gibberella pulicaris*. *Phytochemistry*, **37**, 1001–1005.

Gerber, I.B. and Dubery, I.A. (2004) Protein phosphorylation in *Nicotiana tabacum* cells in response to perception of lipopolysaccharides from *Burkholderia cepacia*. *Phytochemistry*, **65**, 2957–2966.

Gerber, I.B., Laukens, K., Witters, E. and Dubery, I.A. (2006) Identification of lipopolysaccharide-responsive phosphoproteins in *Nicotiana tabacum* cells. *Plant Physiol. Biochem.* **44**, 369–379.

Glazebrook, J., Zook, M., Mert, F., Kagan, I., Rogers, E.E., Crute, R.I., Holub, B.E., Hammerschmidt, R. and Ausubel, M.F. (1997) Phytoalexin-deficient mutants of Arabidopsis reveal that *PAD4* encodes a regulatory factor and that four *PAD* genes contribute to downy mildew resistance. *Genetics*, **146**, 381–392.

Gomès, E., Sagot, E., Gaillard, C., Laquitaine, L., Poinsot, B., Sanejouand, H.Y., Delrot, S. and Coutos-Thévenot, P. (2003) Nonspecific lipid-transfer protein genes expression in grape (*Vitis* sp.) cells in response to fungal elicitor treatments. *Mol. Plant–Microbe Interact.* **16**, 456–464.

Gonzalez-Lamothe, R., Mitchell, G., Gattuso, M., Diarra, M.S., Malouin, F. and Bouarab, K. (2009) Plant antimicrobial agents and their effects on plant and human pathogens. *Int. J. Mol. Sci.* **10**, 3400–3419.

Granado, J., Felix, G. and Boller, T. (1995) Perception of fungal sterols in plants: subnanomolar concentrations of ergosterol elicit extracellular alkalinisation in tomato cells. *Plant Physiol.* **107**, 485–490.

Grayer, R.J. and Kokubun, T. (2001) Plant–fungal interactions: the search for phytoalexins and other antifungal compounds from higher plants. *Phytochemistry*, **56**, 253–263.

Grenier, J. and Asselin, A. (1990) Some pathogenesis-related proteins are chitosanases with lytic activity against fungal spores. *Mol. Plant–Microbe Interact.* **3**, 401–407.

Hammerschmidt, R. (1999) Phytoalexins: what have we learned after 60 years? *Annu. Rev. Phytopathol.* **37**, 285–306.

Harborne, J.B. (2000) Arsenal for survival: secondary plant products. *Taxon*, **49**, 435–449.

He, P., Shan, L. and Sheen, J. (2007) Elicitation and suppression of microbe-associated molecular pattern-triggered immunity in plant–microbe interactions. *Cell. Microbiol.* **9**, 1385–1396.

Henry, G., Thonart, P. and Ongena, M. (2012) PAMPs, MAMPs, DAMPs and others: an update of the diversity of plant immunity elicitors. *Biotechnol., Agron., Soc. Environ.* **16**, 257–268.

Hou, S., Zhang, C., Yang, Y. and Wu, D. (2013) Recent advances in plant immunity: recognition, signaling, response and evolution. *Biol. Plant.* **57**, 11–25.

Hsueh, Y.-W., Gilbert, K., Trandum, C., Zuckermann, M. and Thewalt, J. (2005) The effect of ergosterol on dipalmitoylphosphatidylcholine bilayers: a deuterium NMR and calorimetric study. *Biophys. J.* **88**, 1799–1808.

Huffaker, A., Kaplan, F., Vaughan, M.M., Dafoe, J.N., Ni, X., Rocca, R.J., Alborn, T.H., Teal, E.A.P. and Schmelz, A.E. (2011) Novel acidic sesquiterpenoids constitute a dominant class of pathogen-induced phytoalexins in maize. *Plant Physiol.* **156**, 2082–2097.

Im, Y.J., Raychaudhuri, S., Prinz, W.A. and Hurley, J.H. (2005) Structural mechanism for sterol sensing and transport by OSBP-related proteins. *Nature*, **437**, 154–158.

Iriti, M. and Faoro, F. (2007) Review of innate and specific immunity in plants and animals. *Mycopathologia*, **164**, 57–64.

Ishihama, N., Yamada, R., Yoshioka, M., Katou, S. and Yoshioka, H. (2011) Phosphorylation of the *Nicotiana benthamiana* WRKY8 transcription factor by MAPK functions in the defence response. *Plant Cell*, **23**, 1153–1170.

Jones, J.D.G. and Dangl, J.L. (2006) The plant immune system. *Nature*, **444**, 323–329.

Kader, J.C. (1975) Proteins and the intracellular exchange of lipids: stimulation of phospholipids exchange between mitochondria and microsomal fractions by protein isolated from potato tuber. *Biochim. Biophys. Acta*, **380**, 31–44.

Kader, J.C. (1996) Lipid-transfer proteins in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 627–654.

Kasparovsky, T., Milat, M.L., Humbert, C., Blein, J.P., Havel, L. and Mikes, V. (2003) Elicitation of tobacco cells with ergosterol activates a signal pathway including mobilisation of internal calcium. *Plant Physiol. Biochem.* **41**, 495–501.

Kasparovsky, T., Blein, J.P. and Mikes, V. (2004) Ergosterol elicits an oxidative burst in tobacco cells via phospholipase A2 and protein kinase C signal pathway. *Plant Physiol. Biochem.* **42**, 429–435.

Katagiri, F. and Tsuda, K. (2010) Understanding the plant immune system. *Mol. Plant–Microbe Interact.* **23**, 1531–1536.

Kauss, H. and Jeblick, W. (1996) Influence of salicylic acid on the induction of competence for H_2O_2 elicitation (comparison of ergosterol with other elicitors). *Plant Physiol.* **111**, 755–763.

Keen, N.T. (1990) Gene-for-gene complementarity in plant–pathogen interactions. *Annu. Rev. Genet.* **24**, 447–463.

Knoth, C., Ringler, J., Dangl, J.L. and Euglem, T. (2007) *Arabidopsis* WRKY70 is required for full *RPP4*-mediated disease resistance and basal defence against *Hyaloperonospora parasitica*. *Mol. Plant–Microbe Interact.* **20**, 120–128.

Laquitaine, L., Gomès, E., Françios, J., Marchive, C., Pascal, S., Hamdi, S., Atanassova, R., Delrot, S. and Coutos-Thévenot, P. (2006) Molecular basis of ergosterol-induced protection of grape against *Botrytis cinerea*: induction of Type-I LTP promoter activity, WRK, and stilbene synthase gene expression. *Mol. Plant–Microbe Interact.* **19**, 1103–1112.

Lecourieux, D., Ranjeva, R. and Pugin, A. (2006) Calcium in plant defence-signalling pathways. *New Phytol.* **171**, 249–269.

Lederberg, J. and McCray, A.T. (2001) Ome sweet Omics – A genealogical treasury of words. *Scientist*, **15**, 8.

Li, J., Brader, G., Kariola, T. and Palva, E.T. (2006) WRKY 70 modulates the selection of signalling pathways in plant defense. *Plant J.* **46**, 477–491.

Li, X., Xia, B., Jiang, Y., Wu, Q., Wang, C., He, L., Peng, F. and Wang, R. (2010) A new pathogenesis-related protein, LrPR4, from *Lycoris radiata*, and its antifungal activity against *Magnaporthe grisea*. *Mol. Biol. Rep.* **37**, 995–1001.

Linthorst, H.J., Brederode, F.T., van der Does, C. and Bol, J.F. (1993) Tobacco proteinase inhibitor I genes are locally, but not systemically induced by stress. *Plant Mol. Biol.* **21**, 985–992.

Liu, J. and Nes, W.D. (2009) Steroidal triterpenes: design of substrate-based inhibitors of ergosterol and sitosterol synthesis. *Molecules*, **14**, 4690–4706.

Lochman, J. and Mikes, V. (2006) Ergosterol treatment leads to the expression of a specific set of defence-related genes in tobacco. *Plant Mol. Biol.* **62**, 43–51.

Ma, W. (2011) Roles of Ca^{2+} and cyclic nucleotide gated channel in plant innate immunity. *Plant Sci.* **181**, 342–346.

Madala, N.E., Molinaro, A. and Dubery, I.A. (2011) Deciphering the structural and biological properties of the lipid-A moiety of lipopolysaccharides from *Burkholderia cepacia* strain ASP B 2D, in *Arabidopsis thaliana*. *Glycobiology*, **21**, 184–194.

Madala, N.E., Molinaro, A. and Dubery, I.A. (2012) Distinct carbohydrate and lipid-based molecular patterns within lipopolysaccharides from *Burkholderia cepacia* contribute to defence-associated differential gene expression in *Arabidopsis thaliana*. *Innate Immun.* **18**, 140–154.

Mazzotta, S. and Kemmerling, B. (2011) Pattern recognition in plant innate immunity. *J. Plant Pathol.* **93**, 7–17.

Meijer, E.A., De Vries, S.C., Sterk, P., Gadella, D.W., Writz, K.W. and Hendricks, T. (1993) Characterisation of a nonspecific lipid-transfer protein EP2 from carrot (*Daucus carota* L.). *Mol. Cell. Biochem.* **123**, 159–166.

Mert-Türk, F. (2002) Phytoalexins: defence or just a response to stress? *J. Cell Mol. Biol.* **1**, 1–6.

Mishina, T.E. and Zeier, J. (2007) Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in Arabidopsis. *Plant J.* **50**, 500–513.

Mohd As'wad, A.W., Sariah, M., Paterson, R.R.M., Zainal Abidin, M.A. and Lima, N. (2011) Ergosterol analyses of oil palm seedlings and plants infected with *Ganoderma*. *Crop Prot.* **30**, 1438–1442.

Monaghan, J. and Zipfel, C. (2012) Plant pattern recognition receptor complexes at the plasma membrane. *Curr. Opin. Plant Biol.* **15**, 349–357.

Muthamilarasan, M. and Prasad, M. (2013) Plant innate immunity: an updated insight into defence mechanism. *J. Biosci.* **38**, 1–17.

Narusaka, Y., Shinya, T., Narusaka, M., Motoyama, N., Shimada, H., Murakami, K. and Shibuya, N. (2013) Presence of LYM2 dependent but CERK1 independent disease resistance in *Arabidopsis*. *Plant Signal. Behav.* **8**, e25345.

Naseri, G., Sohani, M.M., Pourmassalehgou, A. and Allahi, S. (2012) In planta transformation of rice (*Oryza sativa*) using thaumatin-like protein gene for enhancing resistance to sheath blight. *J. Biotechnol.* **11**, 7885–7893.

Naumowicz, M., Petelska, A.D. and Figaszewski, Z.A. (2011) Chronopotentiometric studies of phosphatidylcholine bilayers modified by ergosterol. *Steroids*, **76**, 967–973.

Nürnberger, T., Brunner, F., Kemmerling, B. and Piater, L. (2004) Innate immunity in plants and animals: striking similarities and obvious differences. *Immunol. Rev.* **198**, 249–266.

Okazaki, Y. and Saito, K. (2012) Recent advances of metabolomics in plant biotechnology. *Plant Biotechnol. Rep.* **6**, 1–15.

Osman, H., Vauthrin, S., Mikes, V., Milat, M.-A., Panabières, F., Marais, A., Brunie, S., Maume, B., Ponchet, M. and Blein, J.-P. (2001) Mediation of elicitin activity on tobacco is assumed by elicitor–sterol complexes. *Mol. Biol. Cell* **12**, 2825–2834.

Pandey, S.P. and Somssich, I.E. (2009) The role of WRKY transcription factors in plant immunity. *Plant Physiol.* **150**, 1648–1655.

Peck, S.C., Nühse, T.S., Hess, D., Iglesias, A., Meins, F. and Boller, T. (2001) Directed proteomics identifies a plant-specific protein rapidly phosphorylated in response to bacterial and fungal elicitors. *Plant Cell*, **13**, 1467–1475.

Pedras, M.S.C. and Ahiahonu, P.W.K. (2005) Metabolism and detoxification of phytoalexins and analogs by phytopathogenic fungi. *Phytochemistry*, **66**, 391–411.

Peng, X., Hu, Y., Tang, X., Zhou, P., Deng, X., Wang, H. and Guo, Z. (2012) Constitutive expression of rice WRKY30 gene increases the endogenous jasmonic acid accumulation, PR gene expression and resistance to fungal pathogens in rice. *Planta*, **236**, 1485–1498.

Pennisi, E. (2010) Armed and dangerous. *Science*, **327**, 804–805.

Piater, L.A., Nürnberger, T. and Dubery, I.A. (2004) Identification of a lipopolysaccharide responsive erk-like MAP kinase in tobacco leaf tissue. *Mol. Plant Pathol.* **5**, 331–341.

Pieterse, C.M.J., Leon-Reyes, A., Van der Ent, S. and Van Wees, S.C.M. (2009) Networking by small-molecule hormones in plant immunity. *Nat. Chem. Biol.* **5**, 308–316.

Postel, S. and Kemmerling, B. (2009) Plant systems for recognition of pathogen-associated molecular patterns. *Semin. Cell Dev. Biol.* **20**, 1025–1031.

Pritchard, L. and Birch, P. (2011) A systems biology perspective on plant–microbe interactions: biochemical and structural targets of pathogen effectors. *Plant Sci.* **180**, 584–603.

Qi, Y., Tsuda, K., Glazebrook, J. and Katagiri, F. (2011) Physical association of pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) immune receptors in Arabidopsis. *Mol. Plant Pathol.* **12**, 702–708.

Robert, N., Ferran, J., Breda, C., Coutos-Thevenot, P., Boulay, M., Buffard, D. and Esnault, R. (2001) Molecular characterisation of the incompatible interaction of *Vitis vinifera* leaves with *Pseudomonas syringae* pv. *pisi*: expression of genes coding for stilbene synthase and class 10 PR-Protein. *Eur. J. Plant Pathol.* **107**, 249–261.

Robert, N., Roche, K., Lebeau, Y., Breda, C., Bouley, M., Esnault, R. and Buffard, D. (2002) Expression of grapevine chitinase genes in berries and leaves infected by fungal and bacterial pathogens. *Plant Sci.* **162**, 389–400.

Roche, Y., Gerbeau-Pissot, P., Buhot, B., Thomas, D., Bonneau, L., Gresti, J., Mongrand, S., Perrier-Cornet, J.-M. and Simon-Plas, F. (2008) Depletion of phytosterols from the plant plasma membrane provides evidence for disruption of lipid rafts. *FASEB J.* **22**, 3980–3991.

Rossard, S., Luini, E., Pérault, J.M., Bonmort, J. and Roblin, G. (2006) Early changes in membrane permeability, production of oxidative burst and modification of PAL activity induced by ergosterol in cotyledons of *Mimosa pudica*. *J. Exp. Bot.* **57**, 1245–1252.

Rossard, S., Roblin, G. and Atanassova, R. (2010) Ergosterol triggers characteristic elicitation steps in *Beta vulgaris* leaf tissues. *J. Exp. Bot.* **61**, 1807–1816.

Sanabria, N.M., Huang, J.-C. and Dubery, I.A. (2010) Self/nonself perception in plants in innate immunity and defence. *Self/Nonself Immun. Recog.* **1**, 40–54.

Sanabria, N.M., van Heerden, H. and Dubery, I.A. (2012) Molecular characterization and regulation of a *Nicotiana tabacum* S-domain receptor-like kinase gene induced during an early rapid response to lipopolysaccharides. *Gene*, **501**, 39–48.

Schmidt, S.M. and Panstruga, R. (2011) Pathogenomics of fungal plant parasites: what have we learnt about pathogenesis? *Curr. Opin. Plant Biol.* **14**, 392–399.

Schwessinger, B. and Zipfel, C. (2008) News from the frontline: recent insights into PAMP-triggered immunity in plants. *Curr. Opin. Plant Biol.* **11**, 389–395.

Sels, J., Mathys, J., De Coninck, B.M.A., Cammue, B.P.A. and De Bolle, M.F.C. (2008) Plant pathogenesis-related (PR) proteins: a focus on PR peptides. *Plant Physiol. Biochem.* **46**, 941–950.

Sharma, N., Sharma, K.P., Gaur, R.K. and Gupta, V.K. (2011) Role of chitinase in plant defence. *Asian J. Biochem.* **6**, 29–37.

Shimizu, T., Nakano, T., Takamizawa, D., Desaki, Y., Ishii-Minami, N., Nishizawa, Y., Minami, E., Okada, K., Yamane, H., Kaku, H. and Shibuya, N. (2010) Two LysM receptor molecules, CEBiP and OsCERK1 cooperatively regulate chitin elicitor signal in rice. *Plant J.* **64**, 204–214.

Smith, C.J. (1996) Accumulation of phytoalexins: defence mechanism and stimulus response system. *New Phytol.* **132**, 1–45.

Soh, H.C., Park, A.R., Park, S., Back, K., Yoon, J.B., Park, H.G. and Kim, Y.S. (2011) Comparative analysis of pathogenesis-related protein 10 (PR10) genes between fungal resistant and susceptible peppers. *Eur. J. Plant Pathol.* **132**, 37–48.

Sterk, P., Booij, H., Schellekens, G.A., Van Kammen, A. and De Vries, S.C. (1991) Cell-specific expression of the carrot EP2 lipid transfer protein gene. *Plant Cell*, **3**, 907–921.

Stracke, R., Werber, M. and Weisshaar, B. (2001) The R2R3-MYB gene family in *Arabidopsis thaliana*. *Curr. Opin. Plant Biol.* **4**, 447–456.

Sumner, L.W., Mendes, P. and Dixon, R.A. (2003) Plant metabolomics: large-scale phytochemistry in the functional genomics era. *Phytochemistry*, **62**, 817–836.

Thomma, B.P.H., Nelissen, I., Eggermont, K. and Broekaert, W.F. (1999) Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to fungus *Alternaria brassicicola*. *Plant J.* **19**, 163–171.

Thomma, B.P.H., Nürnberger, T. and Joosten, M.H.A.J. (2011) Of PAMPs and effectors: the blurred PTI–ETI dichotomy. *Plant Cell*, **23**, 4–15.

Tierens, K.F.M., Thomma, B.P.H.J., Brouwer, M., Schmidt, J., Kistner, K., Porzel, A., Mauch-Mani, M., Cammue, B.P.A. and Broekaert, W.F. (2001) Study of the role of antimicrobial glucosinolate-derived isothiocyanates in resistance of Arabidopsis to microbial pathogens. *Plant Physiol.* **125**, 1688–1699.

Torres, J.M., Calderón, H., Rodríguez-Arango, E., Morales, J.G. and Arango, R. (2012) Differential induction of pathogenesis-related proteins in banana in response to *Mycosphaerella fijiensis* infection. *Eur. J. Plant Pathol.* **133**, 887–898.

Trigos, Á., Castellanos-Onorio, O., Salinas, A. and Yáñez-Morales, M.J. (2005) Ergosterol from *Phytophthora drechsleri*, an unusual metabolite of a member of this genus. *Mycopathologia*, **159**, 469–471.

Tugizimana, F., Steenkamp, P.A., Piater, L.A. and Dubery, I.A. (2012) Ergosterol-induced sesquiterpenoid synthesis in tobacco cells. *Molecules*, **17**, 1698–1715.

Tugizimana, F., Piater, L.A. and Dubery, I.A. (2013) Plant metabolomics: a new frontier in phytochemical analysis. *S. Afr. J. Sci.* **109**, 1–11.

Tugizimana, F., Steenkamp, P.A., Piater, L.A. and Dubery, I.A. (2014) Multi-platform metabolomic analyses of ergosterol-induced dynamic changes in *Nicotiana tabacum* cells. *PLoS ONE*, **9**, e87846. doi: 10:1371/journal.pone.0087846.

Ülker, B. and Somssich, I.E. (2004) WRKY transcription factors: from DNA binding towards biological function. *Curr. Opin. Plant Biol.* **7**, 491–498.

Van der Biezen, E.A. and Jones, J.D. (1998) Plant disease-resistance proteins and the gene-for-gene concept. *Trends Biochem. Sci.* **23**, 454–456.

Van Loon, L.C. and Van Strien, E.A. (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiol. Mol. Plant Pathol.* **55**, 85–97.

Van Loon, L.C., Rep, M. and Pieterse, C.M.J. (2006) Significance of inducible defence-related proteins in infected plants. *Annu. Rev. Phytopathol.* **44**, 135–162.

Vatsa, P., Chiltz, A., Luini, E., Vandelle, E., Pugin, A. and Roblin, G. (2011a) Cytosolic calcium rises and related events in ergosterol-treated *Nicotiana* cells. *Plant Physiol. Biochem.* **49**, 764–773.

Vatsa, P., Chiltz, A., Bourque, S., Wendehenne, D., Garcia-Brugger, A. and Pugin, A. (2011b) Involvement of putative glutamate receptors in plant defence signaling and NO production. *Biochimie*, **93**, 2095–2101.

Vega, M.A. and Boland, R.L. (1988) Presence of sterol-binding sites in the cytosol of French-bean (*Phaseolus vulgaris*) roots. *Biochem. J.* **250**, 565–569.

Vidhyasekaran, P. (2014) PAMP signals in plant innate immunity: signal perception and transduction. In: *Signaling and Communication in Plants* (Baluška, F., ed.), pp. 17–115, 228. Dordrecht, Heidelberg, New York, London: Springer.

Vigers, A.J., Roberts, W.K. and Selitrennikoff, C.P. (1991) A new family of plant antifungal proteins. *Mol. Plant–Microbe Interact.* **4**, 315–323.

Wanderley-Nogueira, A.C., Belarmino, L.C., Soares-Cavalcanti, N.D.M., Bezerra-Neto, J.P., Kido, E.A., Pandolfi, V. and Abdelnoor, R.V. (2012) An overall evaluation of the Resistance (R) and Pathogenesis-Related (PR) superfamilies in soybean, as compared with *Medicago* and *Arabidopsis*. *Genet. Mol. Biol.* **35**, 260–271.

Xing, T., Ouellet, T. and Miki, B. (2002) Towards genomic and proteomic studies of protein phosphorylation in plant–pathogen interactions. *Trends Plant Sci.* **5**, 224–230.

Xu, X., Bittman, R., Duportail, G., Heissler, D., Vilcheze, C. and London, E. (2001) Effect of the structure of natural sterols and sphingolipids on the formation of ordered sphingolipid/sterol domains (rafts): comparison of cholesterol to plant, fungal, and disease-associated sterols and comparison of sphingomyelin, cerebrosides, and ceramide. *J. Biol. Chem.* **276**, 33 540–33 546.

Yang, X., Deng, F. and Ramonell, K.M. (2012) Receptor-like kinases and receptor-like proteins: keys to pathogen recognition and defense signalling in plant innate immunity. *Front. Biol.* **7**, 155–166.

Zappel, N.F. and Panstruga, R. (2008) Heterogeneity and lateral compartmentalization of plant plasma membrane. *Curr. Opin. Plant Biol.* **11**, 632–640.

Zhang, S. and Klessig, D.F. (2001) MAPK cascades in plant defence signaling. *Trends Plant Sci.* **6**, 520–527.

Zhang, Z., Collinge, D.B. and Thordal-Christensen, H. (1995) Germin-like oxalate oxidase, a H_2O_2-producing enzyme, accumulates in barley attacked by the powdery mildew fungus. *Plant J.* **8**, 139–145.

Zhao, X.R., Lin, Q. and Brookes, P.C. (2005) Does soil ergosterol concentration provide a reliable estimate of soil fungal biomass? *Soil Biol. Biochem.* **37**, 311–317.

Zhou, F., Zhang, Z., Gregersen, P.L., Mikkelsen, J.D., de Neergaard, E., Collinge, D. and Thordal-Christensen, H. (1998) Molecular characterization of the oxalate oxidase involved in the response of barley to the powdery mildew fungus. *Plant Physiol.* **117**, 33–41.

Zhu, F., Xu, M., Wang, S., Jia, S., Zhang, P., Lin, H. and Xi, D. (2012) Prokaryotic expression of pathogenesis-related protein 1 gene from *Nicotiana benthamiana*: antifungal activity and preparation of its polyclonal antibody. *Biotechnol. Lett.* **34**, 919–924.

Zhu, L. and Li, N. (2013) Quantitation, networking, and function of protein phosphorylation in plant cells. *Science*, **3**, 1–7.

Zipfel, C. (2008) Pattern-recognition receptors in plant innate immunity. *Curr. Opin. Immunol.* **20**, 10–16.

Zipfel, C. (2009) Early molecular events in PAMP-triggered immunity. *Curr. Opin. Plant Biol.* **12**, 414–420.

Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E.J., Jones, J.D.G., Felix, G. and Boller, T. (2004) Bacterial disease resistance in *Arabidopsis* through flagellin perception. *Nature*, **428**, 764–767.